THE DIFFERENTIAL EFFECT OF RADIATIONS ON MENDELIAN PHENOTYPES OF THE GOLD-FISH, CARASSIUS AURATUS [1]

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The types of goldfish used in the following experiments are those described in the companion paper by Goodrich and Anderson (1939). These are the common goldfish, the transparent shubunkin, and the hybrid between these two known as the calico shubunkin. Genetic analysis has shown that this is a monohybrid cross and the formulae assigned have been: ordinary goldfish TT, the transparent shubunkin $T'T'$, and the calico fish TT'.

The original purpose of the ultraviolet treatment was to destroy certain parts of the color pattern in the calico fish and to study its regeneration. It was, however, soon discovered that lighter treatment than that needed to destroy the chromatophores apparently induced the formation of new pigmented areas. Consequently a more careful program of experimentation was outlined to verify these preliminary findings.

Methods

The source of illumination has been a small laboratory mercury lamp obtained from the Hanovia Company (their model E). The quartz tube is 16 mm. in diameter, has a length of arc of 50 mm., and operates on 110-volt circuit. For purposes of destruction of melanophores, treatments frequently of 30 minutes or more were administered, but for stimulation of pigment formation most treatments were of 10 minutes duration at distances varying from 2 cm. to 6 cm. from the lamp. Only a small area was irradiated on each fish. Other parts of the body within the zone of illumination were protected. The areas treated varied from about 0.2 to 0.9 sq. cm. in size. These were delimited by pieces of wet filter paper over which were placed pieces of tin foil, which in turn were held in place by more filter paper. Wet cotton was put over the head and the operculum and over the rear of

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the body and caudal fin. This kept the fish moist and helped to hold it in place. The fishes were anesthetized in a 1 per cent urethane solution and were placed on a paraffin block modeled to hold the fishes nearly upright. During irradiation the spot treated was kept wet with distilled water to prevent drying of the tissue. Photographs of both sides of the fish were taken before treatment. The irradiated areas were outlined on the photographic prints and later the location of new spots was marked on these prints, or additional photographs taken if thought desirable. The fish were inspected at weekly intervals for the first three months after the treatment and those fishes that survived were observed at longer intervals for the succeeding six months.

Experiments

After the preliminary experiments, it was first planned to treat approximately equal numbers of the three Mendelian types. Accordingly, ten of each were irradiated. Later, the numbers treated were increased, especially of the hybrid type which was the only form which gave a positive reaction. The final lot of fish irradiated included 24 of the ordinary goldfish TT, 17 of the transparent shubunkin $T'T'$, and 52 of the hybrids TT', giving a total of 93 fish treated.[2] Areas with few or no melanophores were selected for irradiation. The essential result from the comparative study was that the hybrids alone showed a positive reaction by development of new melanophores, while in the two parental types no melanophores were formed. Most fish in all these groups exhibited inflammation and sometimes necrosis of tissues. In the goldfish TT the xanthophores and guanin crystals (of the reflecting tissue) were frequently destroyed. Spots or cell clusters appeared only in the hybrids. These were first observed as small faintly grayish chromatophores, having long delicate processes. The number of cells increased and in about eight weeks these cells became typical mature goldfish melanophores. (Figure 3 shows the inflammation following irradiation, and Figs. 4 and 5 the development of a cell cluster in the same spot.) Figure 1 is a photograph of a hybrid TT' taken on March 29 just before radiation and the area irradiated is outlined. Figure 2 is of the same fish on May 27. Three new spots, one small and two large, have appeared in the radiated area and one outside (in dotted circle). All but one of the new spots

[2] Eight fish of doubtful classification are excluded from these totals. Inspection of pattern indicated that they probably were one normal goldfish and seven transparent shubunkins. All gave negative reactions. Even if the presumed transparent types were transferred to the list of 52 calico shubunkins the essential results as indicated by the graphs, Figs. 6 and 7, would not be altered.

were located in the dermis superficial to the scales. This one exceptional spot was beneath the scales.

The companion paper (Goodrich and Anderson, 1939) has shown that the hybrid or calico fish is characterized by an irregular mottling and, moreover, that this pattern is subject to change during the life of the individual. It therefore seemed possible that the appearance of new spots after radiation might be nothing more than the normal sequence of events. On this account, many more of the hybrids were

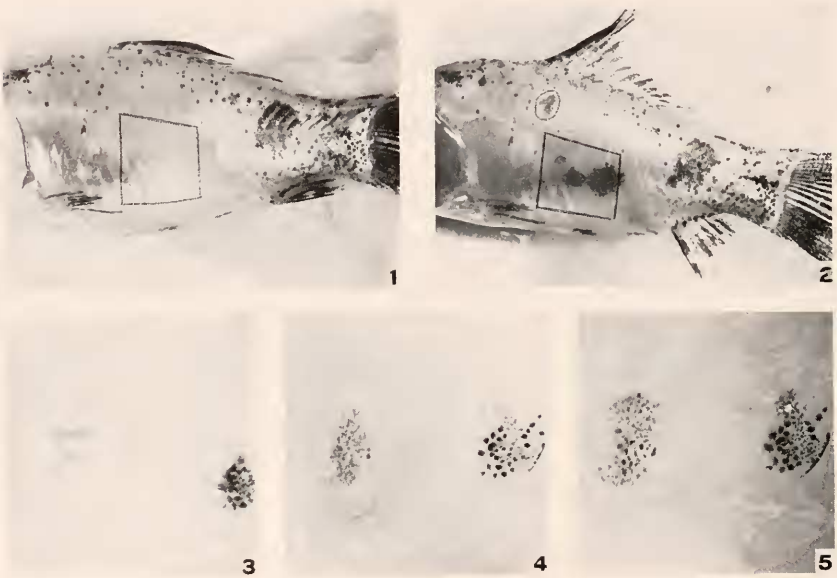

FIG. 1. Photograph of a hybrid TT' taken on March 29 just before radiation. The area later irradiated is outlined with dotted line. × 1¼.

FIG. 2. Photograph of same fish as in Fig. 1 taken on May 27. Three new spots (one small and two large) have appeared in the radiated area and one outside (in dotted circle). × 1¼.

FIGS. 3, 4, 5. Successive photographs of the same area on a hybrid fish. × 6. Irradiation Nov. 13, 1937. Fig. 3, appearance Nov. 27; congestion of capillaries in center (an older spot at right). Fig. 4, Jan. 2, 1938. Fig. 5, Jan. 25, 1938.

treated and the results subjected to analysis. This has shown that the irradiated areas produced a significantly greater number of spots or cell clusters than appeared on non-radiated areas. It was also found that the new spots appeared chiefly from three to six weeks after treatment with the maximum number arising during the fifth week (see charts, Figs. 6 and 7). In 24 cases two or more cell clusters appeared within the radiated area, in 17 cases only one new spot and none were recorded in 11 cases. These results have been compared with the total number of cell clusters appearing on both sides of the body (exclusive of head and fins). The results appear significant even

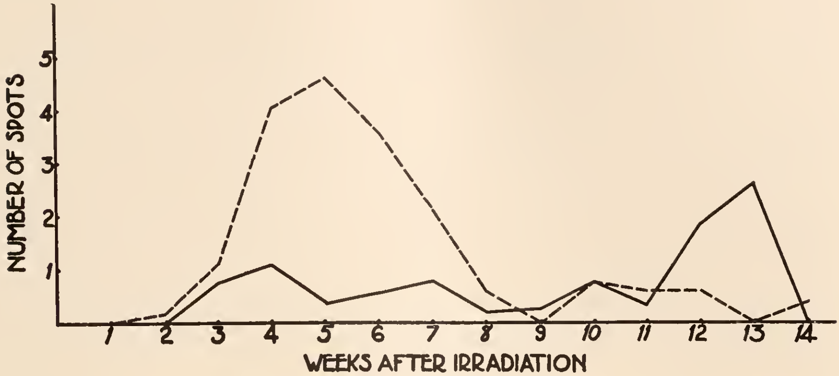

FIG. 6. Graph of numbers of spots X10 appearing in successive weeks after irradiation. Dotted line, irradiated area. Solid line, other parts of body (head and fins not included).

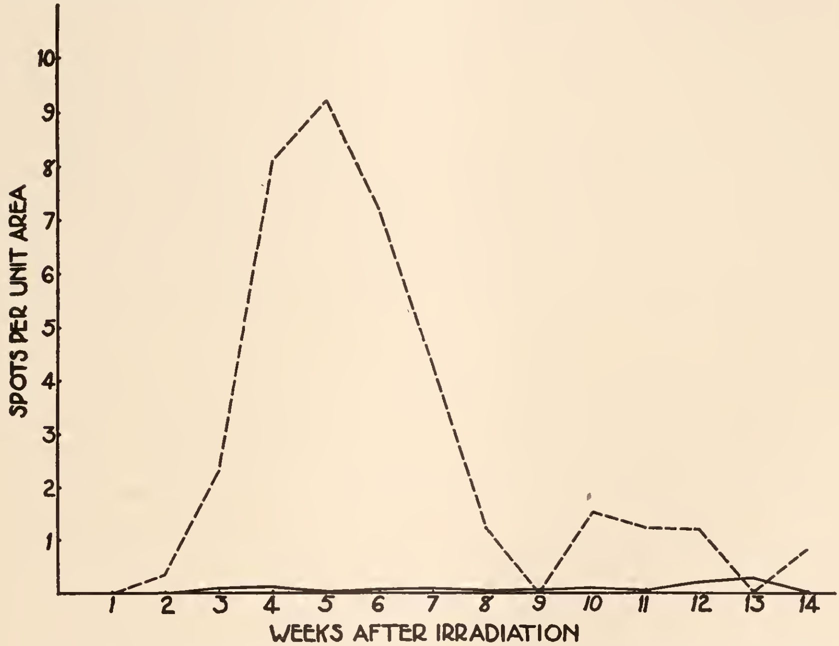

FIG. 7. Graph showing same data as Fig. 6 corrected for relative size of areas. Dotted line, numbers in irradiated area X20. Solid line, numbers on other parts of body X-1.

when no correction is made for the difference in areas compared. When, however, such a comparison is made it is found that the total non-radiated surface examined was approximately twenty times that of the average irradiated area. A graph, incorporating this correction, is shown (Fig. 7) and indicates a notable excess of development of spots in the irradiated areas. During the observational periods, from treatment until 14 weeks thereafter, there appeared a total number of 62 new spots or cell clusters within the irradiated areas and 32 outside of these areas. If we multiply by the factor 20 ($20 \times 62 = 1240$), it appears that had spots appeared at a similar rate in the non-radiated region there would have been 1240 spots, whereas there were only 32. This proportion of nearly 38 : 1 is then an index of the increased reaction of the radiated region. It is not impossible that this is an underestimate. The areas chosen for treatment were frequently below the lateral line, because this region was more clear of melanophores, and it is possible that the ventral region is one having less inherent capacity for production of melanophores.

The new cells recorded in the above experiments were in all respects similar to normal melanophores present elsewhere on the fish. Two sets of subsidiary experiments were carried out which, incidentally, gave further confirmation that these cells were normal melanophores. (1) It was found that the melanophores of the hybrid responded very irregularly to an illuminated white environment. In some cells the pigment became concentrated and in others it remained dispersed. New cells arising in the irradiated areas showed this same variability of reaction. (2) Ten scales bearing new cell clusters were transplanted to other parts of the fish as had previously been done by Goodrich and Nichols (1933) with non-radiated fish. The results were similar. The cells lived and in four cases increased, spreading over adjoining scales.

Discussion

The observations presented in this and the preceding paper (Goodrich and Anderson, 1939) show that the hybrid or calico shubunkin retains the potentiality to produce irregularly situated spots during a considerable part of the life cycle. The radiation appears to stimulate a precocious development of the spots in the areas treated. The question then arises as to what developmental or other conditions control the appearance of these spots or cell clusters. Goodrich (1927), working on the Japanese fish *Oryzias latipes*, suggested that the variegated pattern could be explained by the ameboid migration of predetermined melanoblasts of two types—that producing the maximum amount of melanin and the other such a small amount that they remained virtually colorless. Recent investigations such as those of DuShane (1935) and Twitty (1936) on amphibia have tended to confirm the hypothesis of an early determination of wandering chromatoblasts. The paper by Willier and Rawles (1938) on the chick opens the possibility of cell determination and migration in forms where hormones have been shown to be largely operative in other phases of pigment control. The observations of Apgar (1935) on *Triturus* have suggested the concept of a widespread distribution of colorless chromatoblasts. It, therefore, seems not improbable that we may consider the calico shubunkin (especially Type *B* of the companion paper) to be invisibly spotted during development with colorless chromatoblasts—singly or in nests—and that these multiply and differentiate independently at irregular intervals to form the spots or clusters of melanophores. In some respects this hypothesis resembles the old theory of embryonic cell rests advanced to explain the cause of cancer.

In certain individual fish a wave of destruction takes place, possibly due to some hormone action, which destroys all melanophores and possibly all melanoblasts in the affected areas. We have never observed the appearance of new spots in a region which has suffered such wholesale destruction.

Attention should be called to the production in goldfish of pigment cells by X-rays (Smith, 1932). The cells appeared within a few days after treatment and disappeared a few weeks later. They did not seem to be homologous to the pattern-producing cells and resembled cells that had previously been observed arising after various mechanical injuries to the tissues (Smith, 1931). In our own experiments we have noted three cases of the formation of such cells. They were seen on the normal goldfish *TT* after unusually severe radiation from the mercury arc lamp and the appearance and history of these cells were similar to those noted by Smith.

The contrasting reactions of the three genotypes indicate that the hybrid or calico fish retains in adult condition a far greater potency to produce melanophores than either parental form. Goodrich and Hansen (1931) have pointed out that all three types form melanophores in early development. The ordinary goldfish loses these by wholesale destruction usually at about three months of age, while in the transparent shubunkin relatively few ever appear. Neither of these two parental forms produced typical melanophores when irradiated and it may be suggested that melanoblasts have also been destroyed or are largely absent. In contrast, the heterozygous type retains the melanoblasts.

No attempt is made in this paper to determine what wave-lengths have produced the observed effect. The mercury vapor arc produces a wide range of wave-lengths. The extensive literature on effects of ultraviolet light shows that both stimulating and destructive effects have been observed. Sperti, Loofbourow, and Dwyer (1937), working on yeast cells, have suggested that cells when injured by ultraviolet liberate some growth-promoting substance, thus indicating a possible interrelation of injurious and stimulating effects. The treatments used in our experiments have been relatively more severe than those which have produced primarily stimulating effects on isolated cells. Ultraviolet light penetrates but a few millimeters through animal tissues. Sato (1933) has shown that the ultraviolet light bands characteristic of the mercury arc will pass through fish scales. The effect produced in our experiments may well be due chiefly to the regenerative processes following the inflammation and destruction of tissue.

Summary

1. Radiation from a mercury vapor lamp produced differing reactions in three Mendelian phenotypes. Two parental forms, the ordinary goldfish and the transparent shubunkin, do not develop melanophores as a result of the treatment. The F_1 hybrid, or calico shubunkin, does respond by an acceleration in the production of new spots or clusters of melanophores.

2. It is suggested that the hybrid during development becomes supplied with colorless chromatoblasts throughout the dermis which are stimulated to precocious multiplication and differentiation as a result of the radiation.

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